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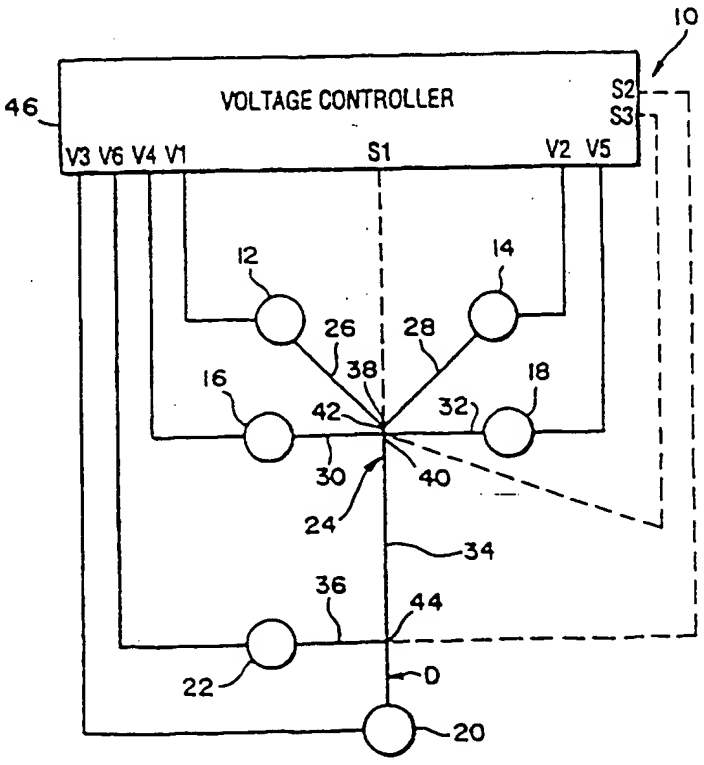
(54) Title: APPARATUS AND METHOD FOR PERFORMING MICROFLUIDIC MANIPULATIONS FOR CHEMICAL ANALYSIS AND SYNTHESIS

(57) Abstract

A microchip loboratory system (10) and method provide fluidic manipulations for a variety of applications, including sample injection for microchip chemical separations. The microchip is fabricated using standard photolithographic procedures and chemical wet etching, with the substrate and cover plate joined using direct bonding. Capillary electrophoresis and electrochromatography are performed in channels (26, 28, 30, 32, 34, 36, 38) formed in the substrate. Analytes are loaded into a four-way intersection of channels by electrokinetically pumping the analyte through the intersection (40), followed by a switching of the potentials to force an analyte plug into the separation channel (34).



Description

APPARATUS AND METHOD FOR PERFORMING MICROFLUIDIC MANIPULATIONS FOR CHEMICAL ANALYSIS AND SYNTHESIS

This invention was made with Government support under contract DE-AC05-84OR21400 awarded by the U.S. Department of Energy to Martin Marietta Energy Systems, Inc. and the Government has certain rights in this invention.

Field of the invention

The present invention relates generally to miniature instrumentation for chemical analysis, chemical sensing and synthesis and, more specifically, to electrically controlled manipulations of fluids in micromachined channels. These manipulations can be used in a variety of applications, including the electrically controlled manipulation of fluid for capillary electrophoresis, liquid chromatography, flow injection analysis, and chemical reaction and synthesis.

Background of the invention

Laboratory analysis is a cumbersome process. Acquisition of chemical and biochemical information requires expensive equipment, specialized labs and highly trained personnel. For this reason, laboratory testing is done in only a fraction of circumstances where acquisition of chemical information would be useful. A large proportion of testing in both research and clinical situations is done with crude manual methods that are characterized by high labor costs, high reagent consumption, long turnaround times, relative imprecision and poor reproducibility. The practice of techniques such as electrophoresis that are in widespread use in biology and medical laboratories have not changed significantly in thirty years.

Operations that are performed in typical laboratory processes include specimen preparation, chemical/biochemical conversions, sample fractionation, signal detection and data processing. To accomplish these tasks, liquids are often measured and dispensed with volumetric accuracy, mixed together, and subjected to one or several different physical or chemical environments that accomplish conversion or fractionation. In research, diagnostic, or development situations, these operations are carried out on a macroscopic scale using fluid volumes in the range of a few microliters to several liters at a time. Individual operations are performed in series, often using different specialized equipment and instruments for separate steps in the process. Complications, difficulty

systems have not met with success. This is primarily because precise manipulation of tiny fluid volumes in extremely narrow channels has proven to be a difficult technological hurdle.

One prominent field susceptible to miniaturization is capillary electrophoresis. Capillary electrophoresis has become a popular technique for separating charged molecular species in solution. The technique is performed in small capillary tubes to reduce band broadening effects due to thermal convection and hence improve resolving power. The small tubes imply that minute volumes of materials, on the order of nanoliters, must be handled to inject the sample into the separation capillary tube.

Current techniques for injection include electromigration and siphoning of sample from a container into a continuous separation tube. Both of these techniques suffer from relatively poor reproducibility, and electromigration additionally suffers from electrophoretic mobility-based bias. For both sampling techniques the input end of the analysis capillary tube must be transferred from a buffer reservoir to a reservoir holding the sample. Thus, a mechanical manipulation is involved. For the siphoning injection, the sample reservoir is raised above the buffer reservoir holding the exit end of the capillary for a fixed length of time.

An electromigration injection is effected by applying an appropriately polarized electrical potential across the capillary tube for a given duration while the entrance end of the capillary is in the sample reservoir. This can lead to sampling bias because a disproportionately larger quantity of the species with higher electrophoretic mobilities migrate into the tube. The capillary is removed from the sample reservoir and replaced into the entrance buffer reservoir after the injection duration for both techniques.

A continuing need exists for methods and apparatuses which lead to improved electrophoretic resolution and improved injection stability.

Summary of the Invention

The present invention provides microchip laboratory systems and methods that allow complex biochemical and chemical procedures to be conducted on a microchip under electronic control. The microchip laboratory systems comprises a material handling apparatus that transports materials through a system of interconnected, integrated channels on a microchip. The movement of the materials is precisely directed by controlling the electric fields produced in the integrated channels. The precise control of the movement of such materials enables precise mixing, separation, and reaction as needed to implement a desired biochemical or chemical procedure.

first intersection and toward the fourth reservoir after a selected volume of material from the first intersection is transported through the second intersection toward the fourth reservoir. Such control can be used to push the analyte plug further down the separation channel while enabling a second analyte plug to be injected through the first intersection.

In another aspect, the microchip laboratory system acts as a microchip flow control system to control the flow of material through an intersection formed by integrated channels connecting at least four reservoirs. The microchip flow control system simultaneously applies a controlled electrical potential to at least three of the reservoirs such that the volume of material transported from the first reservoir to a second reservoir through the intersection is selectively controlled solely by the movement of a material from a third reservoir through the intersection. Preferably, the material moved through the third reservoir to selectively control the material transported from the first reservoir is directed toward the same second reservoir as the material from the first reservoir. As such, the microchip flow control system acts as a valve or a gate that selectively controls the volume of material transported through the intersection. The microchip flow control system can also be configured to act as a dispenser that prevents the first material from moving through the intersection toward the second reservoir after a selected volume of the first material has passed through the intersection. Alternatively, the microchip flow control system can be configured to act as a diluter that mixes the first and second materials in the intersection in a manner that simultaneously transports the first and second materials from the intersection toward the second reservoir.

Other objects, advantages and salient features of the invention will become apparent from the following detailed description, which taken in conjunction with the annexed drawings, discloses preferred embodiments of the invention.

Brief Description of the Drawings

Figure 1 is a schematic view of a preferred embodiment of the present invention;

Figure 2 is an enlarged, vertical sectional view of a channel shown;

Figure 3 is a schematic, top view of a microchip according to a second preferred embodiment of the present invention;

Figure 4 is an enlarged view of the intersection region of Figure 3;

Figure 5 are CCD images of a plug of analyte moving through the intersection of the Figure 30 embodiment;

Figure 17(b) is an electropherogram of rhodamine B and fluorescein with a separation field strength of 1.5 kV/cm and a separation length of 1.6 mm;

Figure 17(c) is an electropherogram of rhodamine B and fluorescein with a separation field strength of 1.5 kV/cm and a separation length of 11.1 mm;

Figure 18 is a graph showing variation of the number of plates per unit time as a function of the electric field strength for rhodamine B at separation lengths of 1.6 mm (circle) and 11.1 mm (square) and for fluorescein at separation lengths of 1.6 mm (diamond) and 11.1 mm (triangle);

Figure 19 shows a chromatogram of coumarins analyzed by electrochromatography using the system of Figure 12;

Figure 20 shows a chromatogram of coumarins resulting from micellar electrokinetic capillary chromatography using the system of Figure 12;

Figures 21(a) and 21(b) show the separation of three metal ions using the system of Figure 12;

Figure 22 is a schematic, top plan view of a microchip according to the Figure 3 embodiment, additionally including a reagent reservoir and reaction channel;

Figure 23 is a schematic view of the embodiment of Figure 20, showing applied voltages;

Figure 24 shows two electropherograms produced using the Figure 22 embodiment;

Figure 25 is a schematic view of a microchip laboratory system according to a sixth preferred embodiment of the present invention;

Figure 26 shows the reproducibility of the amount injected for arginine and glycine using the system of Figure 25;

Figure 27 shows the overlay of three electrophoretic separations using the system of Figure 25;

Figure 28 shows a plot of amounts injected versus reaction time using the system of Figure 25;

Figure 29 shows an electropherogram of restriction fragments produced using the system of Figure 25;

Figure 30 is a schematic view of a microchip laboratory system according to a seventh preferred embodiment of the present invention.

Figure 31 is a schematic view of the apparatus of Figure 21, showing sequential applications of voltages to effect desired fluidic manipulations; and

Figure 32 is a graph showing the different voltages applied to effect the fluidic manipulations of Figure 23.

discussed in more detail below. Each reservoir 12-22 is in fluid communication with a corresponding channel 26, 28, 30, 32, 34, 36, and 38 of the channel system 24. The first channel 26 leading from the first reservoir 12 is connected to the second channel 28 leading from the second reservoir 14 at a first intersection 38. Likewise, the third channel 30 from the third reservoir 16 is connected to the fourth channel 32 at a second intersection 40. The first intersection 38 is connected to the second intersection 40 by a reaction chamber or channel 42. The fifth channel 34 from the fifth reservoir 20 is also connected to the second intersection 40 such that the second intersection 40 is a four-way intersection of channels 30, 32, 34, and 42. The fifth channel 34 also intersects the sixth channel 36 from the sixth reservoir 22 at a third intersection 44

The materials stored in the reservoirs preferably are transported electrokinetically through the channel system 24 in order to implement the desired analysis or synthesis. To provide such electrokinetic transport, the laboratory system 10 includes a voltage controller 46 capable of applying selectable voltage levels, including ground. Such a voltage controller can be implemented using multiple voltage dividers and multiple relays to obtain the selectable voltage levels. The voltage controller is connected to an electrode positioned in each of the six reservoirs 12-22 by voltage lines V1-V6 in order to apply the desired voltages to the materials in the reservoirs. Preferably, the voltage controller also includes sensor channels S1, S2, and S3 connected to the first, second, and third intersections 38, 40, 44, respectively, in order to sense the voltages present at those intersections.

The use of electrokinetic transport on microminiaturized planar liquid phase separation devices, described above, is a viable approach for sample manipulation and as a pumping mechanism for liquid chromatography. The present invention also entails the use of electroosmotic flow to mix various fluids in a controlled and reproducible fashion. When an appropriate fluid is placed in a tube made of a correspondingly appropriate material, functional groups at the surface of the tube can ionize. In the case of tubing materials that are terminated in hydroxyl groups, protons will leave the surface and enter an aqueous solvent. Under such conditions the surface will have a net negative charge and the solvent will have an excess of positive charges, mostly in the charged double layer at the surface. With the application of an electric field across the tube, the excess cations in solution will be attracted to the cathode, or negative electrode. The movement of these positive charges through the tube will drag the solvent with them. The steady state velocity is given by equation 1,

$$v = \frac{\varepsilon \xi E}{4 \pi \eta} \qquad (1)$$

volts per centimeter using present techniques of providing insulating layers), which may provide insufficient electrokinetic movement.

The channel pattern 24 is formed in a planar surface of the substrate using standard photolithographic procedures followed by chemical wet etching. The channel pattern may be transferred onto the substrate with a positive photoresist (Shipley 1811) and an e-beam written chrome mask (Institute of Advanced Manufacturing Sciences, Inc.). The pattern may be chemically etched using HF/NH_4F solution

After forming the channel pattern, a cover plate may then be bonded to the substrate using a direct bonding technique whereby the substrate and the cover plate surfaces are first hydrolyzed in a dilute NH_4OH/H_2O_2 solution and then joined. The assembly is then annealed at about 500° C in order to insure proper adhesion of the cover plate to the substrate.

Following bonding of the cover plate, the reservoirs are affixed to the substrate, with portions of the cover plate sandwiched therebetween, using epoxy or other suitable means. The reservoirs can be cylindrical with open opposite axial ends. Typically, electrical contact is made by placing a platinum wire electrode in each reservoirs. The electrodes are connected to a voltage controller 46 which applies a desired potential to select electrodes, in a manner described in more detail below.

A cross section of the first channel is shown in Figure 2 and is identical to the cross section of each of the other integrated channels. When using a non-crystalline material (such as glass) for the substrate, and when the channels are chemically wet etched, an isotropic etch occurs, *i.e.*, the glass etches uniformly in all directions, and the resulting channel geometry is trapezoidal. The trapezoidal cross section is due to "undercutting" by the chemical etching process at the edge of the photoresist. In one embodiment, the channel cross section of the illustrated embodiment has dimensions of 5.2 μm in depth, 57 μm in width at the top and 45 μm in width at the bottom. In another embodiment, the channel has a depth "d" of 10μm, an upper width "w1" of 90μm, and a lower width "w2" of 70μm.

An important aspect of the present invention is the controlled electrokinetic transportation of materials through the channel system 24. Such controlled electrokinetic transport can be used to dispense a selected amount of material from one of the reservoirs through one or more intersections of the channel structure 24. Alternatively, as noted above, selected amounts of materials from two reservoirs can be transported to an intersection where the materials can be mixed in desired concentrations.

potential of the first reservoir 12A or the potentials at reservoirs 16A and/or 18A, can be floated momentarily to provide the flow shown by the short dashed arrows in Figure 4. Under these conditions, the primary flow will be from the first reservoir 12A down towards the separation channel waste reservoir 20A. The flow from the second and third reservoirs 16A, 18A will be small and could be in either direction. This condition is held long enough to transport a desired amount of material from the first reservoir 12A through the intersection 40A and into the separation channel 34A. After sufficient time for the desired material to pass through the intersection 40A, the voltage distribution is switched back to the original values to prevent additional material from the first reservoir 12A from flowing through the intersection 40A toward the separation channel 34A.

One application of such a "gated dispenser" is to inject a controlled, variable-sized plug of analyte from the first reservoir 12A for electrophoretic or chromatographic separation in the separation channel 34A. In such a system, the first reservoir 12A stores analyte, the second reservoir 16A stores an ionic buffer, the third reservoir 18A is a first waste reservoir and the fourth reservoir 20A is a second waste reservoir. To inject a small variable plug of analyte from the first reservoir 12A, the potentials at the buffer and first waste reservoirs 16A, 18A are simply floated for a short period of time (≈ 100 ms) to allow the analyte to migrate down the separation column 34A. To break off the injection plug, the potentials at the buffer reservoir 16A and the first waste reservoir 18A are reapplied. Alternatively, the valving sequence could be effected by bringing reservoirs 16A and 18A to the potential of the intersection 40A and then returning them to their original potentials. A shortfall of this method is that the composition of the injected plug has an electrophoretic mobility bias whereby the faster migrating compounds are introduced preferentially into the separation column 34A over slower migrating compounds.

In Figure 5, a sequential view of a plug of analyte moving through the intersection of the Figure 3 embodiment can be seen by CCD images The analyte being pumped through the laboratory system 10A was rhodamine B (shaded area), and the orientation of the CCD images of the injection cross or intersection is the same as in Figure 3. The first image, (A), shows the analyte being pumped through the injection cross or intersection toward the first waste reservoir 18A prior to the injection. The second image, (B), shows the analyte plug being injected into the separation column 34A. The third image, (C), depicts the analyte plug moving away from the injection intersection after an injection plug has been completely introduced into the separation column 34A. The potentials at the buffer and first waste reservoirs 16A, 18A were floated for 100 ms while the sample moved into the separation column 34A. By the time

in the first and second channels 26A, 30A need to be maintained higher than the potential of the intersection 40A during mixing. Such potentials will cause the materials from the first and second reservoirs 12A and 16A to simultaneously move through the intersection 40A and thereby mix the two materials. The potentials applied at the first and second reservoirs 12A, 16A can be adjusted as desired to achieve the selected concentration of each material. After dispensing the desired amounts of each material, the potential at the second reservoir 16A may be increased in a manner sufficient to prevent further material from the first reservoir 12A from being transported through the intersection 40A toward the third reservoir 30A.

Analyte Injector

Shown in Figure 6 is a microchip analyte injector 10B according to the present invention. The channel pattern 24B has four distinct channels 26B, 30B, 32B, and 34B micromachined into a substrate 49 as discussed above. Each channel has an accompanying reservoir mounted above the terminus of each channel portion, and all four channels intersect at one end in a four way intersection 40B. The opposite ends of each section provide termini that extend just beyond the peripheral edge of a cover plate 49' mounted on the substrate 49. The analyte injector 10B shown in Figure 6 is substantially identical to the gated dispenser 10A except that the electrical potentials are applied in a manner that injects a volume of material from reservoir 16B through the intersection 40B rather than from the reservoir 12B and the volume of material injected is controlled by the size of the intersection.

The embodiment shown in Figure 6 can be used for various material manipulations. In one application, the laboratory system is used to inject an analyte from an analyte reservoir 16B through the intersection 40B for separation in the separation channel 34B. The analyte injector 10B can be operated in either "load" mode or a "run" mode. Reservoir 16B is supplied with an analyte and reservoir 12B with buffer. Reservoir 18B acts as an analyte waste reservoir, and reservoir 20B acts as a waste reservoir.

In the "load" mode, at least two types of analyte introduction are possible. In the first, known as a "floating" loading, a potential is applied to the analyte reservoir 16B with reservoir 18B grounded. At the same time, reservoirs 12B and 20B are floating, meaning that they are neither coupled to the power source, nor grounded.

The second load mode is "pinched" loading mode, wherein potentials are simultaneously applied at reservoirs 12B, 16B, and 20B, with reservoir 18B grounded in order to control the injection plug shape as discussed in more detail below. As used

The two modes of loading were tested for the analyte introduction into the separation channel 34B. The analyte was placed in the analyte reservoir 16B, and in both injection schemes was "transported" in the direction of reservoir 18B, a waste reservoir. CCD images of the two types of injections are depicted in Figures 8(a)-8(c). Figure 8(a) schematically shows the intersection 40B, as well as the end portions of channels.

The CCD image of Figure 8(b) is of loading in the pinched mode, just prior to being switched to the run mode. In the pinched mode, analyte (shown as white against the dark background) is pumped electrophoretically and electroosmotically from reservoir 16B to reservoir 18B (left to right) with buffer from the buffer reservoir 12B (top) and the waste reservoir 20B (bottom) traveling toward reservoir 18B (right). The voltages applied to reservoirs 12B, 16B, 18B, and 20B were 90%, 90%, 0, and 100%, respectively, of the power supply output which correspond to electric field strengths in the corresponding channels of 400, 270, 690 and 20 V/cm, respectively. Although the voltage applied to the waste reservoir 20B is higher than voltage applied to the analyte reservoir 18B, the additional length of the separation channel 34B compared to the analyte channel 30B provides additional electrical resistance, and thus the flow from the analyte buffer 16B into the intersection predominates. Consequently, the analyte in the injection cross or intersection 40B has a trapezoidal shape and is spatially constricted in the channel 32B by this material transport pattern.

Figure 8(c) shows a floating mode loading. The analyte is pumped from reservoir 16B to 18B as in the pinched injection except no potential is applied to reservoirs 12B and 20B. By not controlling the flow of mobile phase (buffer) in channel portions 26B and 34B, the analyte is free to expand into these channels through convective and diffusive flow, thereby resulting in an extended injection plug.

When comparing the pinched and floating injections, the pinched injection is superior in three areas: temporal stability of the injected volume, the precision of the injected volume, and plug length. When two or more analytes with vastly different mobilities are to be analyzed, an injection with temporal stability insures that equal volumes of the faster and slower moving analytes are introduced into the separation column or channel 34B. The high reproducibility of the injection volume facilitates the ability to perform quantitative analysis. A smaller plug length leads to a higher separation efficiency and, consequently, to a greater component capacity for a given instrument and to higher speed separations.

To determine the temporal stability of each mode, a series of CCD fluorescence images were collected at 1.5 second intervals starting just prior to the

elements of Figure 1. The microchip laboratory system 10C is similar to laboratory systems 10, 10A, and 10B described previously, in that an injection cross or intersection 40C is provided. In the Figure 10 embodiment, a second intersection 64 and two additional reservoirs 60 and 62 are also provided to overcome the problems with reversing the flow in the separation channel.

Like the previous embodiments, the analyte injector system 10C can be used to implement an analyte separation by electrophoresis or chromatography or dispense material into some other processing element. In the laboratory system 10C, the reservoir 12C contains separating buffer, reservoir 16C contains the analyte, and reservoirs 18C and 20C are waste reservoirs. Intersection 40C preferably is operated in the pinched mode as in the embodiment shown in Figure 6. The lower intersection 64, in fluid communication with reservoirs 60 and 62, are used to provide additional flow so that a continuous buffer stream can be directed down towards the waste reservoir 20C and, when needed, upwards toward the injection intersection 40C. Reservoir 60 and attached channel 56 are not necessary, although they improve performance by reducing band broadening as a plug passes the lower intersection 64. In many cases, the flow from reservoir 60 will be symmetric with that from reservoir 62.

Figure 11 is an enlarged view of the two intersections 40C and 64. The different types of arrows show the flow directions at given instances in time for injection of a plug of analyte into the separation channel. The solid arrows show the initial flow pattern where the analyte is electrokinetically pumped into the upper intersection 40C and "pinched" by material flow from reservoirs 12C, 60, and 62 toward this same intersection. Flow away from the injection intersection 40C is carried to the analyte waste reservoir 18C. The analyte is also flowing from the reservoir 16C to the analyte waste reservoir 18C. Under these conditions, flow from reservoir 60 (and reservoir 62) is also going down the separation channel 34C to the waste reservoir 20C. Such a flow pattern is created by simultaneously controlling the electrical potentials at all six reservoirs.

A plug of the analyte is injected through the injection intersection 40C into the separation channel 34C by switching to the flow profile shown by the short dashed arrows. Buffer flows down from reservoir 12C to the injection intersection 40C and towards reservoirs 16C, 18C, and 20C. This flow profile also pushes the analyte plug toward waste reservoir 20C into the separation channel 34C as described before. This flow profile is held for a sufficient length of time so as to move the analyte plug past the lower intersection 64. The flow of buffer from reservoirs 60 and 62 should be low as indicated by the short arrow and into the separation channel 34C to minimize distortion.

separation of rhodamine B (less retained) and sulforhodamine (more retained) using the following conditions: E_{inj} = 400 V/cm, E_{run} = 150 V/cm, buffer = 50 mM sodium tetraborate at pH 9.2. The CCD images demonstrate the separation process at 1 second intervals, with Figure 13(a) showing a schematic of the section of the chip imaged, and with Figures 13(b)-13(e) showing the separation unfold.

Figure 13(b) again shows the pinched injection with the applied voltages at reservoirs 12D, 16D, and 20D equal and reservoir 18D grounded. Figures 13(c)-13(e) shows the plug moving away from the intersection at 1, 2, and 3 seconds, respectively, after switching to the run mode. In Figure 13(c), the injection plug is migrating around a 90° turn, and band distortion is visible due to the inner portion of the plug traveling less distance than the outer portion. By Figure 13(d), the analytes have separated into distinct bands, which are distorted in the shape of a parallelogram. In Figure 13(e), the bands are well separated and have attained a more rectangular shape, *i.e.*, collapsing of the parallelogram, due to radial diffusion, an additional contribution to efficiency loss.

When the switch is made from the load mode to the run mode, a clean break of the injection plug from the analyte stream is desired to avoid tailing. This is achieved by pumping the mobile phase or buffer from channel 26D into channels 30D, 32D, and 34D simultaneously by maintaining the potential at the intersection 40D below the potential of reservoir 12D and above the potentials of reservoirs 16D, 18D, and 20D.

In the representative experiments described herein, the intersection 40D was maintained at 66% of the potential of reservoir 12D during the run mode. This provided sufficient flow of the analyte back away from the injection intersection 40D down channels 30D and 32D without decreasing the field strength in the separation channel 34D significantly. Alternate channel designs would allow a greater fraction of the potential applied at reservoir 12D to be dropped across the separation channel 34D, thereby improving efficiency.

This three way flow is demonstrated in Figures 13(c)-13(e) as the analytes in channels 30D and 32D (left and right, respectively) move further away from the intersection with time. Three way flow permits well-defined, reproducible injections with minimal bleed of the analyte into the separation channel 34D.

Detectors

In most applications envisaged for these integrated microsystems for chemical analysis or synthesis it will be necessary to quantify the material present in a channel at one or more positions similar to conventional laboratory measurement

disadvantage of relatively poor sensitivity. Sensitivity has been increased through surface enhanced Raman spectroscopy (SERS) effects but only at the research level. Electrical or electrochemical detection approaches are also of particular interest for implementation on microchip devices due to the ease of integration onto a microfabricated structure and the potentially high sensitivity that can be attained. The most general approach to electrical quantification is a conductometric measurement, *i.e.*, a measurement of the conductivity of an ionic sample. The presence of an ionized analyte can correspondingly increase the conductivity of a fluid and thus allow quantification. Amperiometric measurements imply the measurement of the current through an electrode at a given electrical potential due to the reduction or oxidation of a molecule at the electrode. Some selectivity can be obtained by controlling the potential of the electrode but it is minimal. Amperiometric detection is a less general technique than conductivity because not all molecules can be reduced or oxidized within the limited potentials that can be used with common solvents. Sensitivities in the 1 nM range have been demonstrated in small volumes (10 nL). The other advantage of this technique is that the number of electrons measured (through the current) is equal to the number of molecules present. The electrodes required for either of these detection methods can be included on a microfabricated device through a photolithographic patterning and metal deposition process. Electrodes could also be used to initiate a chemiluminescence detection process, *i.e.*, an excited state molecule is generated via an oxidation-reduction process which then transfers its energy to an analyte molecule, subsequently emitting a photon that is detected.

Acoustic measurements can also be used for quantification of materials but have not been widely used to date. One method that has been used primarily for gas phase detection is the attenuation or phase shift of a surface acoustic wave (SAW). Adsorption of material to the surface of a substrate where a SAW is propagating affects the propagation characteristics and allows a concentration determination. Selective sorbents on the surface of the SAW device are often used. Similar techniques may be useful in the devices described herein.

The mixing capabilities of the microchip laboratory systems described herein lend themselves to detection processes that include the addition of one or more reagents. Derivatization reactions are commonly used in biochemical assays. For example, amino acids, peptides and proteins are commonly labeled with dansylating reagents or o-phthaldialdehyde to produce fluorescent molecules that are easily detectable. Alternatively, an enzyme could be used as a labeling molecule and reagents, including substrate, could be added to provide an enzyme amplified detection scheme.

an ionic strength that is less than that of the running buffer. Buffer is transported by electroosmosis from the buffer reservoir 16D towards both the analyte waste and waste reservoirs 18D, 20D. This buffer stream prevents the analyte from bleeding into the separation channel 34D. Within a representative embodiment, the relative potentials at the buffer, analyte, analyte waste and waste reservoirs are 1. 0.9, 0.7 and 0, respectively. For 1 kV applied to the microchip, the field strengths in the buffer, analyte, analyte waste, and separation channels during the separation are 170, 130, 180, and 120 V/cm, respectively.

To inject the analyte onto the separation channel 34D, the potential at the buffer reservoir 16D is floated (opening of the high voltage switch) for a brief period of time (0.1 to 10 s), and analyte migrates into the separation channel. For 1 kV applied to the microchip, the field strengths in the buffer, sample, sample waste, and separation channels during the injection are 0, 240, 120, and 110 V/cm, respectively. To break off the analyte plug, the potential at the buffer reservoir 16D is reapplied (closing of a high voltage switch). The volume of the analyte plug is a function of the injection time, electric field strength, and electrophoretic mobility.

The separation buffer and analyte compositions can be quite different, yet with the gated injections the integrity of both the analyte and buffer streams can be alternately maintained in the separation channel 34D to perform the stacking operation. The analyte stacking depends on the relative conductivity of the separation buffer to analyte, γ. For example, with a 5 mM separation buffer and a 0.516 mM sample (0.016 mM dansyl-lysine and 0.5 mM sample buffer), γ is equal to 9.7. Figure 14 shows two injection profiles for didansyl-lysine injected for 2 s with γ equal to 0.97 and 9.7. The injection profile with $\gamma = 0.97$ (the separation and sample buffers are both 5 mM) shows no stacking. The second profile with $\gamma = 9.7$ shows a modest enhancement of 3.5 for relative peak heights over the injection with $\gamma = 0.97$. Didansyl-lysine is an anion, and thus stacks at the rear boundary of the sample buffer plug. In addition to increasing the analyte concentration, the spatial extent of the plug is confined. The injection profile with $\gamma = 9.7$ has a width at half-height of 0.41 s, while the injection profile with $\gamma = 0.97$ has a width at half-height of 1.88 s. The electric field strength in the separation channel 34D during the injection (injection field strength) is 95% of the electric field strength in the separation channel during the separation (separation field strength). These profiles are measured while the separation field strength is applied. For an injection time of 2 s, an injection plug width of 1.9 s is expected for $\gamma = 0.97$.

The concentration enhancement due to stacking was evaluated for several sample plug lengths and relative conductivities of the separation buffer and analyte. The

at (a) 3.3 cm, (b) 9.9 cm, and (c) 16.5 cm from the point of injection for rhodamine B (less retained) and sulforhodamine (more retained). These were taken using the following conditions: injection type was pinched, E_{inj} = 500V/cm, E_{sep} = 170 V/cm, buffer = 50 mM sodium tetraborate at pH 9.2. To obtain electropherograms in the conventional manner, single point detection with the helium-neon laser (green line) was used at different locations down the axis of the separation channel 34D.

An important measure of the utility of a separation system is the number of plates generated per unit time, as given by the formula

$$N/t = L/(Ht)$$

where N is the number of theoretical plates, t is the separation time, L is the length of the separation column, and H is the height equivalent to a theoretical plate. The plate height, H, can be written as

$$H = A + B/u$$

where A is the sum of the contributions from the injection plug length and the detector path length, B is equal to $2D_m$, where D_m is the diffusion coefficient for the analyte in the buffer, and u is the linear velocity of the analyte.

Combining the two equations above and substituting $u = \mu E$ where μ is the effective electrophoretic mobility of the analyte and E is the electric field strength, the plates per unit time can be expressed as a function of the electric field strength:

$$N/t = (\mu E)^2 / (A\mu E + B)$$

At low electric field strengths when axial diffusion is the dominant form of band dispersion, the term $A\mu E$ is small relative to B and consequently, the number of plates per second increases with the square of the electric field strength.

As the electric field strength increases, the plate height approaches a constant value, and the plates per unit time increases linearly with the electric field strength because B is small relative to $A\mu E$. It is thus advantageous to have A as small as possible, a benefit of the pinched injection scheme.

The efficiency of the electrophorectic separation of rhodamine B and sulforhodamine at ten evenly spaced positions was monitored, each constituting a separate experiment. At 16.5 cm from the point of injection, the efficiencies of rhodamine B and sulforhodamine are 38,100 and 29,000 plates, respectively.

experimental data and the calculated numbers. for rhodamine B at the 11.1 mm separation length. This is primarily due to experimental error.

Electrochromatography

A problem with electrophoresis for general analysis is its inability to separate uncharged species. All neutral species in a particular sample will have zero electrophoretic mobility, and thus, the same migration time. The microchip analyte injector 10D shown in Figure 12 can also be used to perform electrochromatography to separate non-ionic analytes. To perform such electrochromatography, the surface of the separation channel 34D was prepared by chemically bonding a reverse phase coating to the walls of the separation channel after bonding the cover plate to the substrate to enclose the channels. The separation channel was treated with 1 M sodium hydroxide and then rinsed with water. The separation channel was dried at 125°C for 24 hours while purging with helium at a gauge pressure of approximately 50 kPa. A 25% (w/w) solution of chlorodimethyloctaldecylsilane (ODS, Aldrich) in toluene was loaded into the separation channel with an over pressure of helium at approximately 90 kPa. The ODS/ toluene mixture was pumped continuously into the column throughout the 18 hour reaction period at 125°C. The channels are rinsed with toluene and then with acetonitrile to remove the unreacted ODS. The laboratory system 10D was used to perform electrochromatography on an analytes composed of coumarin 440 (C440), coumarin 450 (C450) and coumarin 460 (C460; Exciton Chemical Co., Inc.) at 10 μM for the direct fluorescent measurements of the separations and 1 μM for the indirect fluorescent measurements of the void time. A sodium tetraborate buffer (10 mM, pH 9.2) with 25% (v/v) acetonitrile was the buffer.

The analyte injector 10D was operated under a pinched analyte loading mode and a separation (run) mode as described above with respect to Figure 6. The analyte is loaded into the injection cross via a frontal chromatogram traveling from the analyte reservoir 16D to the analyte waste reservoir 18D, and once the front of the slowest analyte passes through the injection intersection 40D, the sample is ready to be analyzed. To switch to the separation mode, the applied potentials are reconfigured, for instance by manually throwing a switch. After switching the applied potentials, the primary flow path for the separation is from the buffer reservoir 12D to the waste reservoir 20D. In order to inject a small analyte plug into the separation channel 34D and to prevent bleeding of the excess analyte into the separation channel, the analyte and the analyte waste reservoirs 16D, 18D are maintained at 57% of the potential applied to

The microchip laboratory system 10D was operated in the "pinched injection" mode described previously. The voltages applied to the reservoirs are set to either loading mode or a "run" (separation) mode. In the loading mode, a frontal chromatogram of the solution in the analyte reservoir 16D is pumped electroosmotically through the intersection and into the analyte waste reservoir 18D. Voltages applied to the buffer and waste reservoirs also cause weak flows into the intersection from the sides, and then into the analyte waste reservoir 18D. The chip remains in this mode until the slowest moving component of the analyte has passed through the intersection 40D. At this point, the analyte plug in the intersection is representative of the analyte solution, with no electrokinetic bias.

An injection is made by switching the chip to the "run" mode which changes the voltages applied to the reservoirs such that buffer now flows from the buffer reservoir 12D through the intersection 40D into the separation channel 34D toward the waste reservoir 20D. The plug of analyte that was in the intersection 40D is swept into the separation channel 34D. Proportionately lower voltages are applied to the analyte and analyte waste reservoirs 16D, 18D to cause a weak flow of buffer from the buffer reservoir 12D into these channels. These flows ensure that the sample plug is cleanly "broken off" from the analyte stream, and that no excess analyte leaks into the separation channel during the analysis.

The results of the MECC analysis of a mixture of C440, C450, and C460 are shown in Figure 20. The peaks were identified by individual analyses of each dye. The migration time stability of the first peak, C440, with changing methanol concentration was a strong indicator that this dye did not partition into the micelles to a significant extent. Therefore it was considered an electroosmotic flow marker with migration time t0. The last peak, C460, was assumed to be a marker for the micellar migration time, tm. Using these values of t0 and tm from the data in Figure 20, the calculated elution range, t0/tm, is 0.43. This agrees well with a literature value of t0/tm = 0.4 for a similar buffer system, and supports our assumption. These results compare well with conventional MECC performed in capillaries and also shows some advantages over the electrochromatography experiment described above in that efficiency is retained with retention ratio. Further advantages of this approach to separating neutral species is that no surface modification of the walls is necessary and that the stationary phase is continuously refreshed during experiments.

4a. In Figure 4b, 0.48, 0.23, and 0.59 fmol of Zn, Cd, and Al, respectively, are injected onto the separation column. The average reproducibility of the amounts injected is 1.6% rsd (percent relative standard deviation) as measured by peak areas (6 replicate analyses). The stability of the laser used to excite the complexes is ≈ 1% rsd. The detection limits are in a range where useful analyses can be performed.

Post-Separation Channel Reactor

An alternate microchip laboratory system 10E is shown in Figure 22. The five-port pattern of channels is disposed on a substrate 49E and with a cover slip 49E', as in the previously-described embodiments. The microchip laboratory system 10E embodiment was fabricated using standard photolithographic, wet chemical etching, and bonding techniques. A photomask was fabricated by sputtering chrome (50 nm) onto a glass slide and ablating the channel design into the chrome film via a CAD/CAM laser ablation system (Resonetics, Inc.). The channel design was then transferred onto the substrates using a positive photoresist. The channels were etched into the substrate in a dilute HF/NH_4F bath. To form the separation channel 34E, a coverplate was bonded to the substrate over the etched channels using a direct bonding technique. The surfaces were hydrolyzed in dilute NH_4OH/H_2O_2 solution, rinsed in deionized, filtered H_2, joined and then annealed at 500°C. Cylindrical glass reservoirs were affixed on the substrate using RTV silicone (made by General Electric). Platinum electrodes provided electrical contact from the voltage controller 46E (Spellman CZE1000R) to the solutions in the reservoirs.

The channel 26E is in one embodiment 2.7 mm in length from the first reservoir 12E to the intersection 40E, while the channel 30E is 7.0 mm, and the third channel 32E is 6.7 mm. The separation channel 34E is modified to be only 7.0 mm in length, due to the addition of a reagent reservoir 22E which has a reagent channel 36E that connects to the separation channel 34E at a mixing tee 44E. Thus, the length of the separation channel 34E is measured from the intersection 40E to the mixing tee 44E. The channel 56 extending from the mixing tee 44E to the waste reservoir 20E is the reaction column or channel, and in the illustrated embodiment this channel is 10.8 mm in length. The length of the reagent channel 36E is 11.6 mm.

In a representative example, the Figure 22 embodiment was used to separate an analyte and the separation was monitored on-microchip via fluorescence using an argon ion laser (351.1 nm, 50 mW, Coherent Innova 90) for excitation. The fluorescence signal was collected with a photomultiplier tube (PMT, Oriel 77340) for point detection and a charge coupled device (CCD, Princeton Instruments, Inc.

point detection scheme at distances of 6 mm and 8 mm from the injection cross, or 1 mm upstream and 1 mm downstream from the mixing tee. This provided information on the effects of the mixing of the two streams.

The electric field strengths in the reagent column and the separation column were approximately equal, and the field strength in the reaction column was twice that of the separation column. This configuration of the applied voltages allowed an approximately 1:1 volume ratio of derivatizing reagent and effluent from the separation column. As the field strengths increased, the degree of turbulence at the mixing tee increased. At the separation distance of 6 mm (1mm upstream from the mixing tee), the plate height as expected as the inverse of the linear velocity of the analyte. At the separation distance of 8 mm (1 mm upstream from the mixing tee), the plate height data decreased as expected as the inverse of the velocity of the analyze. At the separation distance of 8 mm (1 mm downstream from the mixing tee), the plate height data decreases from 140 V/cm to 280 V/cm to 1400 V/cm- This behavior is abnormal and demonstrates a band broadening phenomena when two streams of equal volumes converge. The geometry of the mixing tee was not optimized to minimize this band distortion. Above separation field strength of 840 V/cm, the system stabilizes and again the plate height decreases with increasing linear velocity. For E_{sep} = 1400 V/cm, the ratio of the plate heights at the 8 mm and 6 mm separation lengths is 1.22 which is not an unacceptable loss in efficiency for the separation.

The intensity of the fluorescence signal generated from the reaction of OPA with an amino acid was tested by continuously pumping glycine down the separation channel to mix with the OPA at the mixing tee. The fluorescence signal from the OPA/amino acid reaction was collected using a CCD as the product moved downstream from the mixing tee. Again, the relative volume ratio of the OPA and glycine streams was 1.125. OPA has a typical half-time of reaction with amino acids of 4 s. The average residence times of an analyte molecule in the window of observation are 4.68, 2.34, 1.17, and 0.58 s for the electric field strengths in the reaction column (E_{rxn}) of 240, 480, 960, and 1920 V/cm, respectively. The relative intensities of the fluorescence correspond qualitatively to this 4 s half-time of reaction. As the field strength increases in the reaction channel, the slope and maximum of the intensity of the fluorescence shifts further downstream because the glycine and OPA are swept away from the mixing tee faster with higher field strengths. Ideally, the observed fluorescence from the product would have a step function of a response following the mixing of the separation effluent and derivatizing reagent. However, the kinetics of the reaction and a finite rate of mixing dominated by diffusion prevent this from occurring.

electrophoretic separations with an integrated post-separation reaction channel 56 in a single monolithic device enabling extremely low volume exchanges between individual channel functions.

Pre-Separation Channel Reaction System

Instead of the post-separation channel reactor design shown in Figure 22, the microchip laboratory system 10F shown in Figure 25 includes a pre-separation channel reactor. The pre-separation channel reactor design shown in Figure 25 is similar to that shown in Figure 1, except that the first and second channels 26F, 28F form a "goal-post" design with the reaction chamber 42F rather than the "Y" design of Figure 1. The reaction chamber 42F was designed to be wider than the separation channel 34F to give lower electric field strengths in the reaction chamber and thus longer residence times for the reagents. The reaction chamber is 96 μm wide at half-depth and 6.2 μm deep, and the separation channel 34F is 31 μm wide at half-depth and 6.2 μm deep.

The microchip laboratory system 10F was used to perform on-line pre-separation channel reactions coupled with electrophoretic analysis of the reaction products. Here, the reactor is operated continuously with small aliquots introduced periodically into the separation channel 34F using the gated dispenser discussed above with respect to Figure 3. The operation of the microchip consists of three elements: the derivatization of amino acids with o-phthaldialdehyde (OPA), injection of the sample onto the separation column, and the separation/ detection of the components of the reactor effluent. The compounds used for the experiments were arginine (0.48 mM), glycine (0.58 mM), and OPA (5.1 mM ; Sigma Chemical Co.). The buffer in all of the reservoirs was 20 mM sodium tetraborate with 2% (v/v) methanol and 0.5% (v/v) 2-mercaptoethanol. 2-mercaptoethanol is added to the buffer as a reducing agent for the derivatization reaction.

To implement the reaction the reservoirs 12F, 14F, 16F, 18F, and 20F were simultaneously given controlled voltages of .5 HV, .5 HV, HV, .2 HV, and ground, respectively. This configuration allowed the lowest potential drop across the reaction chamber 42F (25 V/cm for 1.0 kV applied to the microchip) and highest across the separation channel 34F (300 V/cm for 1.0 kV applied to the microchip) without significant bleeding of the product into the separation channel when using the gated injection scheme. The voltage divider used to establish the potentials applied to each of the reservoirs had a total resistance of 100 MΩ with 10 MΩ divisions. The analyte from the first reservoir 12F and the reagent from the second reservoir 14F are

20 fmol of the amino acids injected onto the separation channel 34F. The gated injector allows rapid sequential injections to be made. In this particular case, an analysis could be performed every 4 s. The observed electrophoretic mobilities for the compounds are determined by a linear fit to the variation of the linear velocity with the separation field strength. The slopes were 29.1 and 13.3 $mm^2/(kV\text{-}as)$ for arginine and glycine, respectively. No evidence of Joule heating was observed as indicated by the linearity of the velocity versus field strength data. A linear fit produced correlation coefficients of 0.999 for arginine and 0.996 for glycine for separation field strengths from 0.2 to 2.0 kV/cm.

With increasing potentials applied to the microchip laboratory system 10F, the field strengths in the reaction chamber 42F and separation channel 34F increase. This leads to shorter residence times of the reactants in the reaction chamber and faster analysis times for the products. By varying the potentials applied to the microchip, the reaction kinetics can be studied. The variation in amount of product generated with reaction time is plotted in Figure 28. The response is the integrated area of the peak corrected for the residence time in the detector observation window and photobleaching of the product. The offset between the data for the arginine and the glycine in Figure 28 is due primarily to the difference in the amounts injected, i.e. different electrophoretic mobilities, for the amino acids. A ten-fold excess of OPA was used to obtain pseudo-first order reaction conditions. The slopes of the lines fitted to the data correspond to the rates of the derivatization reaction. The slopes are 0.13 s^{-1} for arginine and 0.11 s^{-1} for glycine corresponding to half-times of reaction of 5.1 and 6.2 s, respectively. These half-times of reaction are comparable to the 4 s previously reported for alanine. We have found no previously reported data for arginine or glycine.

These results show the potential power of integrated microfabricated systems for performing chemical procedures. The data presented in Figure 28 can be produced under computer control within five approximately five minutes consuming on the order of 100 nL of reagents. These results are unprecedented in terms of automation, speed and volume for chemical reactions.

DNA Analysis

To demonstrate a useful biological analysis procedure, a restriction digestion and electrophoretic sizing experiment are performed sequentially on the integrated biochemical reactor/electrophoresis microchip system 10G shown in Figure 29. The microchip laboratory system 10G is identical to the laboratory system shown in Figure 25 except that the separation channel 34G of the laboratory system 10G

placed in the waste 1 reservoir 18G. This enables the cations to propagate into the reaction chamber countercurrent to the DNA and enzyme during the loading of the reaction chamber. The digestion is performed statically by removing all electrical potentials after loading the reaction chamber 42G due to the relatively short transit time of the DNA through the reaction chamber.

Following the digestion period, the products are migrated into the separation channel 34F for analysis by floating the voltages to the buffer and waste 1 reservoirs 16F, 18F. The injection has a mobility bias where the smaller fragments are injected in favor of the larger fragments. In these experiments the injection plug length for the 75- base pair (bp) fragment is estimated to be 0.34 mm whereas for the 1632-bp fragment only 0.22 mm. These plug lengths correspond to 34% and 22% of the reaction chamber volume, respectively. The entire contents of the reaction chamber 42F cannot be analyzed under current separation conditions because the contribution of the injection plug length to the plate height would be overwhelming.

Following digestion and injection onto the separation channel 34F, the fragments are resolved using 1.0% (w/v) hydroxyethyl cellulose as the sieving medium. Figure 30 shows an electropherogram of the restriction fragments of the plasmid pBR322 following a 2 min digestion by the enzyme Hinf I. To enable efficient on-column staining of the double-stranded DNA after digestion but prior to interrogation, the intercalating dye, TOTO-1 (1 μM), is placed in the waste 2 reservoir 20G only and migrates countercurrent to the DNA. As expected, the relative intensity of the bands increases with increasing fragment size because more intercalation sites exist in the larger fragments. The unresolved 220/221 and 507/511-bp fragments having higher intensities than adjacent single fragment peaks due to the band overlap. The reproducibility of the migration times and injection volumes are 0.55 and 3.1 % relative standard deviation (%rsd), respectively, for 5 replicate analyses.

This demonstration of a microchip laboratory system 10G that performs plasmid DNA restriction fragment analysis indicates the possibility of automating and miniaturizing more sophisticated biochemical procedures. This experiment represents the most sophisticated integrated microchip chemical analysis device demonstrated to date. The device mixes a reagent with an analyte, incubates the analyte/reagent mixture, labels the products, and analyzes the products entirely under computer control while consuming 10,000 times less material than the typical small volume laboratory procedure.

In general, the present invention can be used to mix different fluids contained in different ports or reservoirs. This could be used for a liquid

a given reservoir. The field strength can be calculated from the applied voltage and the characteristics of the channel. In addition, the resistance or conductance of the fluid in the channels must also be known.

The resistance of a channel is given by equation 2 where R is the resistance, κ is the resistivity, L is the length of the channel, and A is the cross-sectional area.

$$R = \frac{\kappa L}{A} \qquad (2)$$

Fluids are usually characterized by conductance which is just the reciprocal of the resistance as shown in equation 3. In equation 3, K is the electrical conductance, ρ is the conductivity, A is the cross-sectional area, and L is the length as above.

$$K = \frac{\rho A}{L} \qquad (3)$$

Using ohms law and equations 2 and 3 we can write the field strength in a given channel, i, in terms of the voltage drop across that channel divided by its length which is equal to the current, I_i through channel i times the resistivity of that channel divided by the cross-sectional area as shown in equation 4.

$$E = \frac{V_i}{L} = \frac{IR}{L} = \frac{I_i}{A_i}\frac{\kappa}{\rho A} \qquad (4)$$

Thus, if the channel is both dimensionally and electrically characterized, the voltage drop across the channel or the current through the channel can be used to determine the solvent velocity or flow rate through that channel as expressed in equation 5. It is also noted that fluid flow depends on the zeta potential of the surface and thus on the chemical make-ups of the fluid and surface.

$$V_i \propto I_i \propto \text{Flow}$$

function of time so that the proportions of solvents 1 and 2 are changed from a dominance of solvent 1 to mostly solvent 2. This is shown in Figure 31(c). The latter monotonic change in applied voltage effects the gradient elution liquid chromatography experiment. As the isolated components pass the reagent addition channel 36, appropriate reaction can take place between this reagent and the isolated material to form a detectable species.

Figure 32 shows how the voltages to the various reservoirs are changed for a hypothetical gradient elution experiment. The voltages shown in this diagram only indicate relative magnitudes and not absolute voltages. In the loading mode of operation, static voltages are applied to the various reservoirs. Solvent flow from all reservoirs except the reagent reservoir 22 is towards the analyte waste reservoir 18. Thus, the analyte reservoir 18 is at the lowest potential and all the other reservoirs are at higher potential. The potential at the reagent reservoir should be sufficiently below that of the waste reservoir 20 to provide only a slight flow towards the reagent reservoir. The voltage at the second solvent reservoir 14 should be sufficiently great in magnitude to provide a net flow towards the injection intersection 40, but the flow should be a low magnitude.

In moving to the run (start) mode depicted in Figure 31(b), the potentials are readjusted as indicated in Figure 32. The flow now is such that the solvent from the solvents reservoirs 12 and 14 is moving down the separation channel 34 towards the waste reservoir 20. There is also a slight flow of solvent away from the injection intersection 40 towards the analyte and analyte waste reservoirs 16 and 18 and an appropriate flow of reagent from the reagent reservoir 22 into the separation channel 34. The waste reservoir 20 now needs to be at the minimum potential and the first solvent reservoir 12 at the maximum potential. All other potentials are adjusted to provide the fluid flow directions and magnitudes as indicated in Figure 31(b). Also, as shown in Figure 32, the voltages applied to the solvent reservoirs 12 and 14 are monotonically changed to move from the conditions of a large mole fraction of solvent 1 to a large mole fraction of solvent 2.

At the end of the solvent programming run, the device is now ready to switch back to the inject condition to load another sample. The voltage variations shown in Figure 32 are only to be illustrative of what might be done to provide the various fluid flows in Figures 31(a)-(c). In an actual experiment some to the various voltages may well differ in relative magnitude.

Claims

1. A microchip laboratory system for analyzing or synthesizing chemical material, comprising:

a body having integrated channels connecting a plurality of reservoirs, wherein at least five of the reservoirs simultaneously have a controlled electrical potential associated therewith, such that material from at least one of the reservoirs is transported through the channels toward at least one of the other reservoirs to provide exposure to one or more selected chemical or physical environments, thereby resulting in the synthesis or analysis of the chemical material.

2. The system of claim 1 wherein the material transported is a fluid.

3. The system of claim 1, further comprising:

a first intersection of channels connecting at least three of the reservoirs; and

means for mixing materials from two of the reservoirs at the first intersection.

4. The system of claim 3 wherein the mixing means includes means for producing an electrical potential at the first intersection that is less than the electrical potential at each of the two reservoirs from which the materials to be mixed originate.

5. The system of claim 1, further comprising:

a first intersection of channels connecting first, second, third, and fourth reservoirs; and

means for controlling the volume of a first material transported from the first reservoir to the second reservoir through the first intersection by transporting a second material from the third reservoir through the first intersection.

6. The system of claim 5 wherein the controlling means includes means for transporting the second material through the first intersection toward the second and fourth reservoirs.

7. The system of claim 5 wherein the controlling means includes dispensing means for transporting the second material through the first intersection in a manner that prevents

forming a first intersection wherein at least three of the reservoirs simultaneously have a controlled electrical potential associated therewith such that the volume of material transported from a first reservoir to a second reservoir through the first intersection is selectively controlled solely by the movement of a material from a third reservoir through the first intersection toward another reservoir.

15. The system of claim 14 wherein the material transported is a fluid.

16. The system of claim 14, further comprising:

controlling means for transporting the second material from the third reservoir through the first intersection toward the second reservoir.

17. The system of claim 16 wherein the controlling means includes dispensing means for transporting the second material through the first intersection in a manner that prevents the first material from moving through the first intersection toward the second reservoir after a selected volume of the first material has passed through the first intersection toward the second reservoir.

18. The system of claim 16 wherein the controlling means includes diluting means for mixing the first and second materials in the first intersection in a manner that simultaneously transports the first and second materials from the first intersection toward the second reservoir.

19. The system of claim 14 wherein the integrated channels include a first channel connecting the first and second reservoirs, a second channel connecting the third reservoir with a fourth reservoir in a manner that forms a first intersection with the first channel, and a third channel that connects a fifth reservoir with the second channel at a location between the first intersection and the fourth reservoir.

20. The system of claim 19, further comprising:

mixing means for mixing material from the fifth reservoir with material transported from the first intersection toward the fourth reservoir.

21. The system of claim 19 wherein the third channel crosses the second channel

after a selected time period, simultaneously applying an electrical potential to each of the four reservoirs in a manner that inhibits the movement of the first material through the intersection toward the third reservoir.

FIG. 3

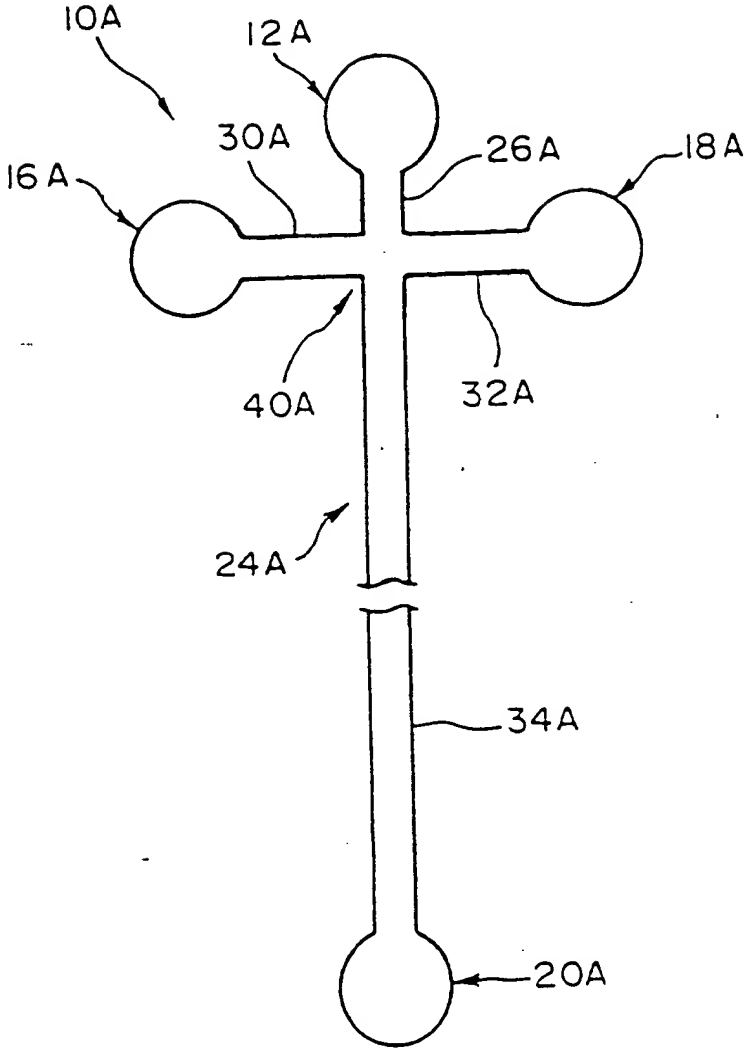


FIG. 4

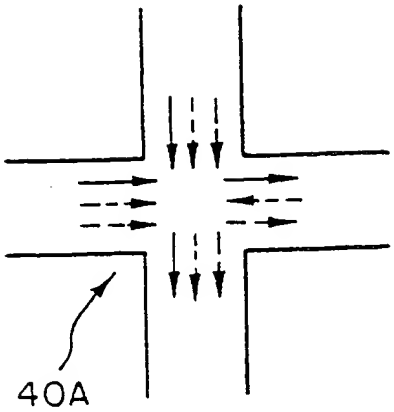


FIG. 6

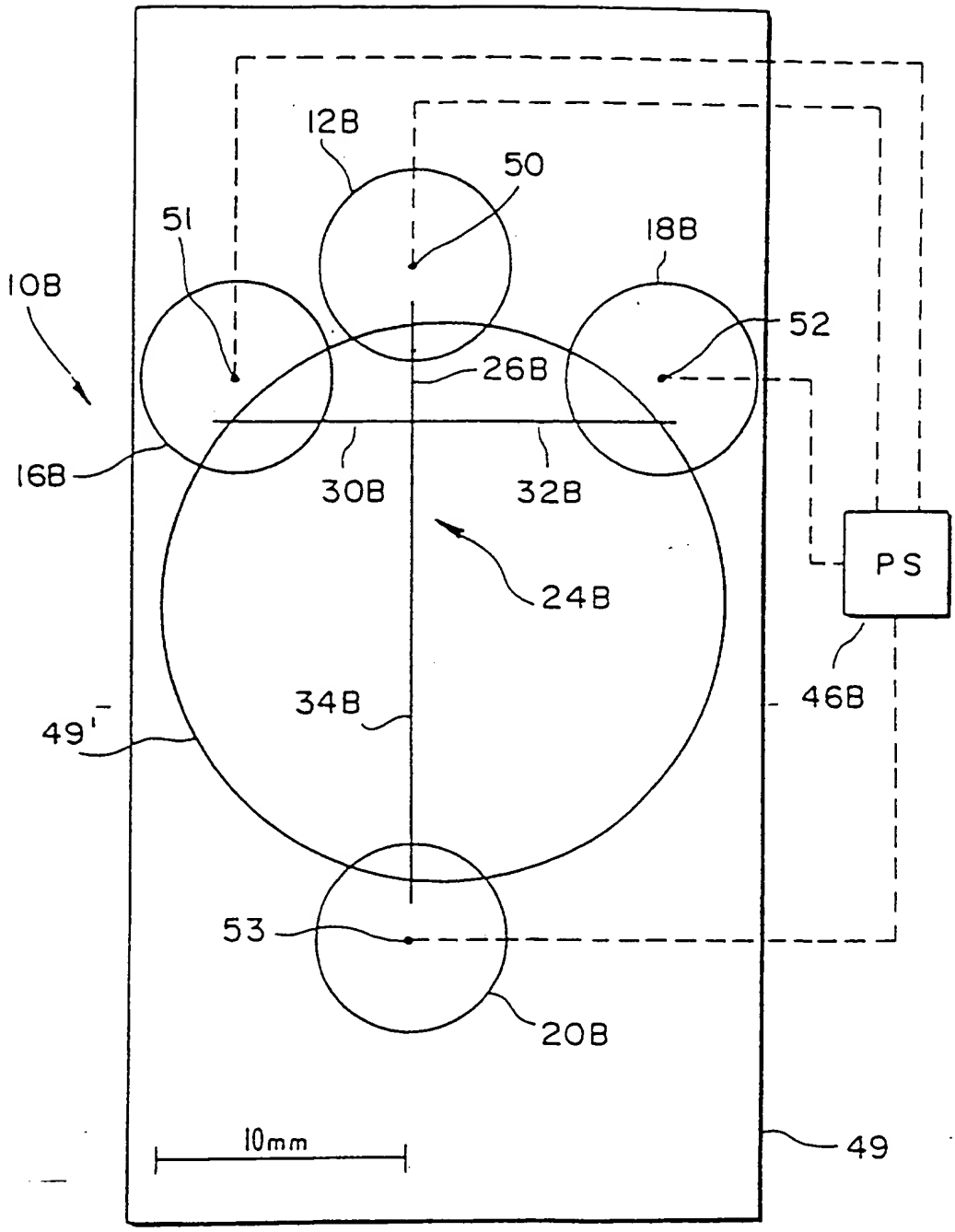

12B
50
51
18B
10B
52
26B
16B
30B
32B
PS
24B
34B
46B
49'
53
20B
10mm
49

FIG. 9

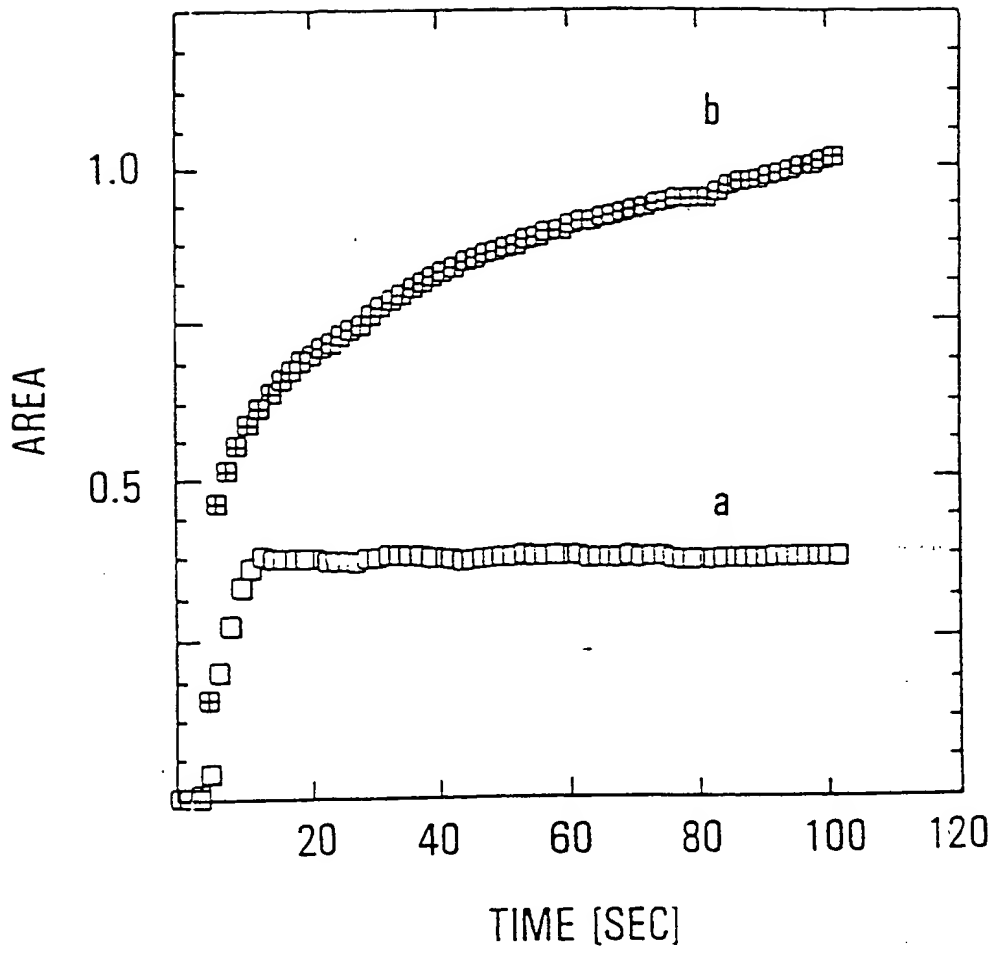


FIG. 12

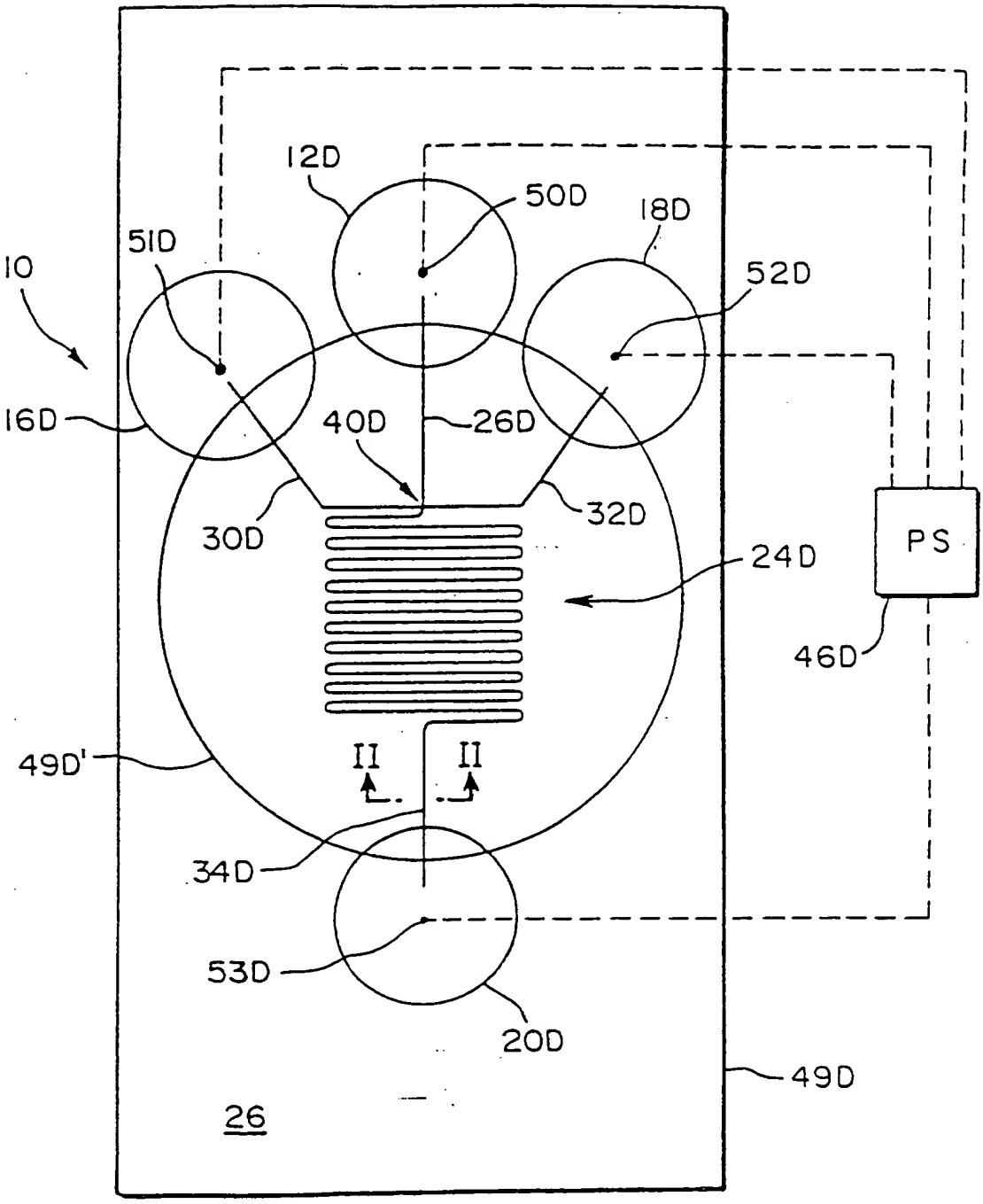

12D
50D
18D
51D
10
52D
16D
40D
26D
30D
32D
24D
PS
46D
49D'
II
II
34D
53D
20D
49D
26

FIG. 14

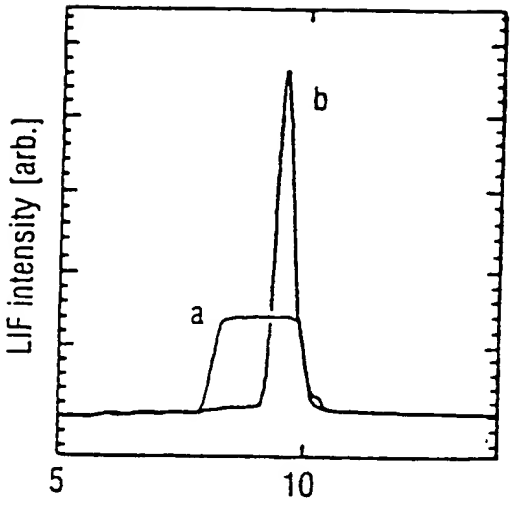


FIG. 15

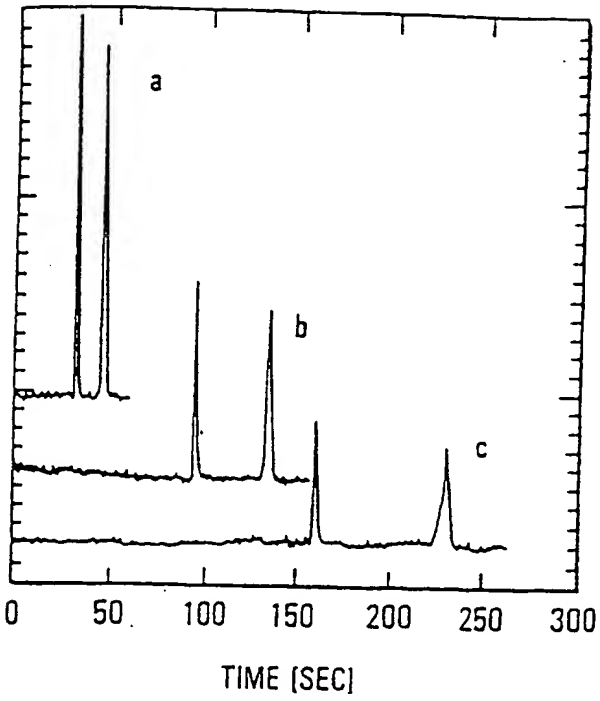


FIG. 16

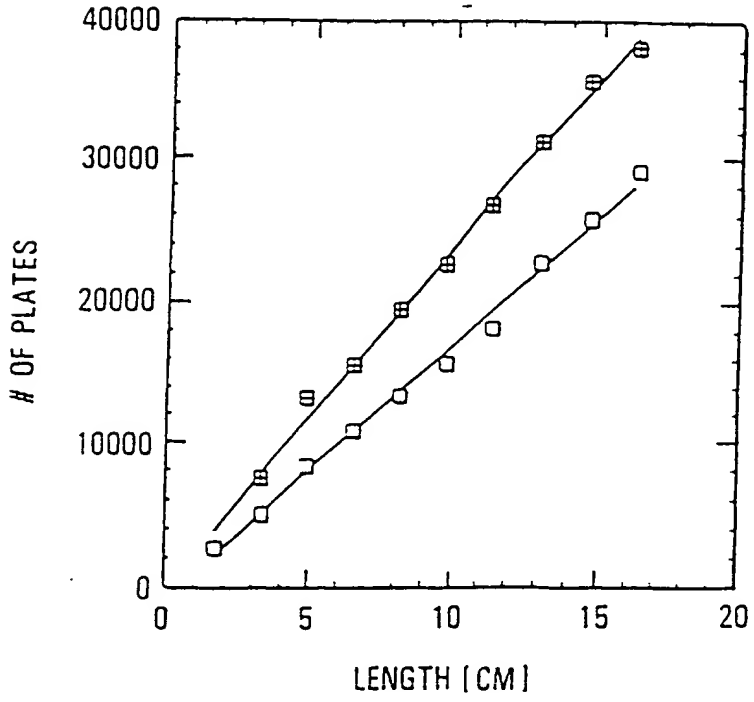


FIG. 17(c)

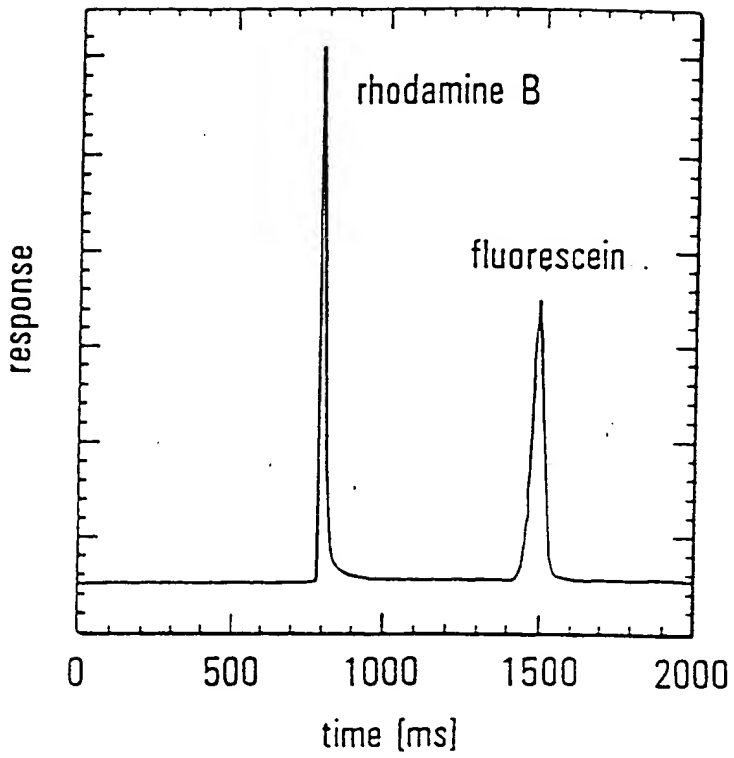


FIG. 18

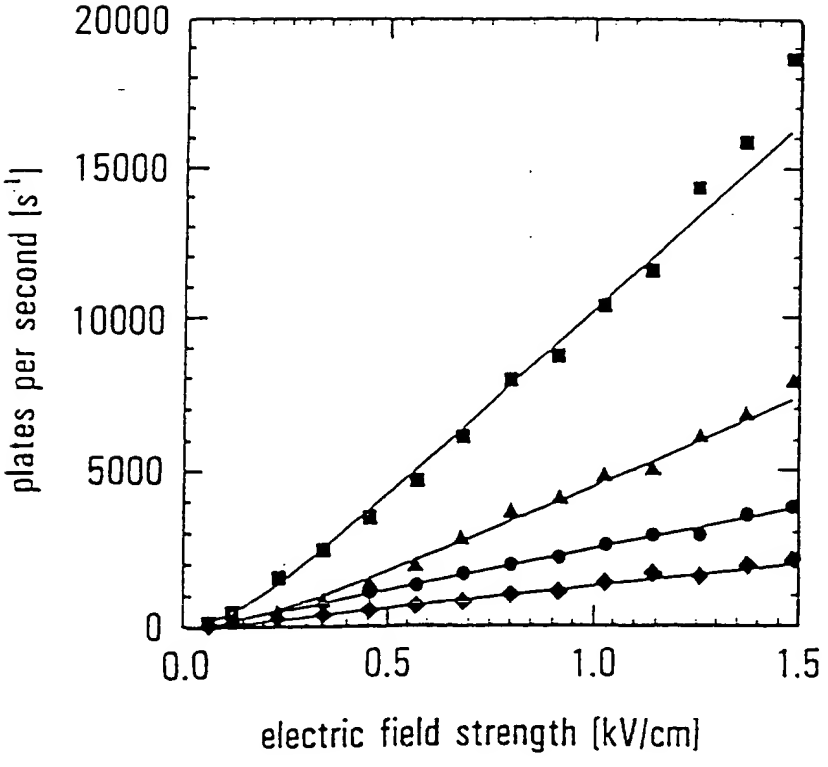


FIG. 21(a)

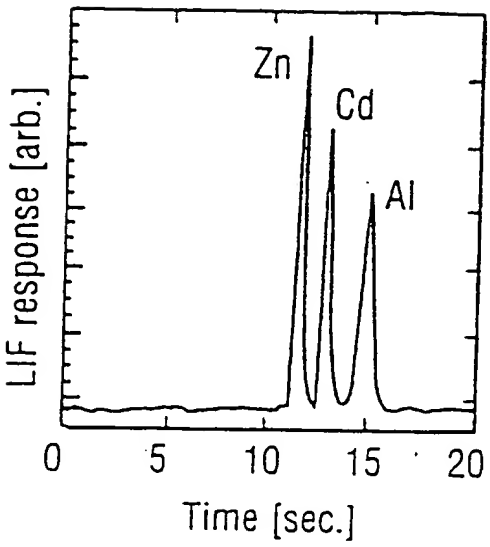


FIG. 21(b)

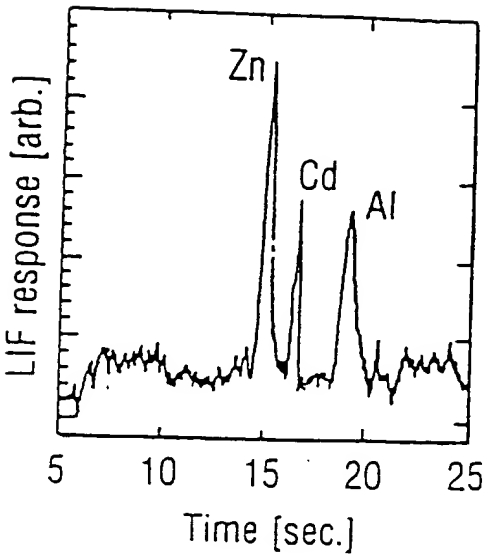


FIG. 24

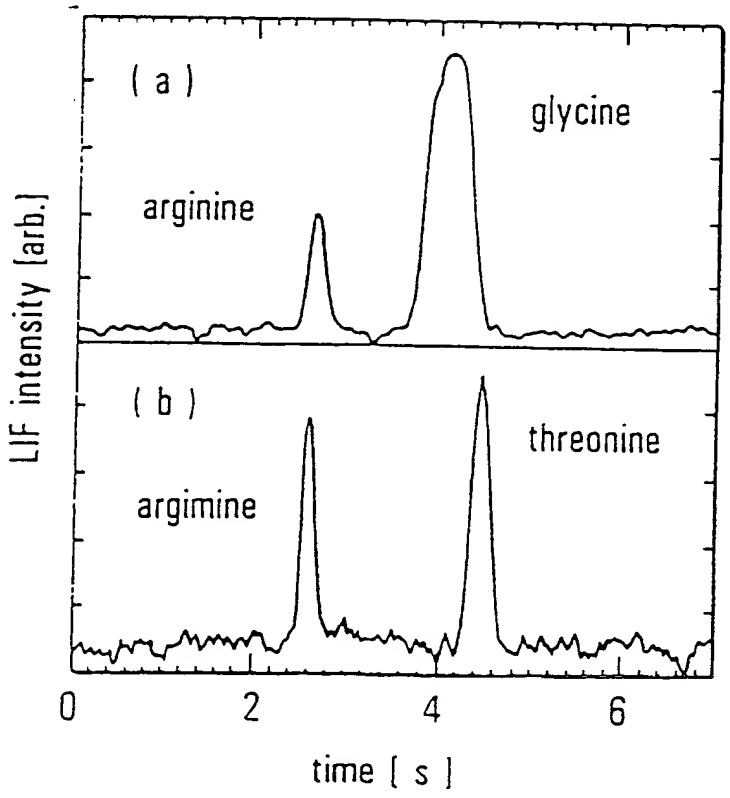


FIG. 25

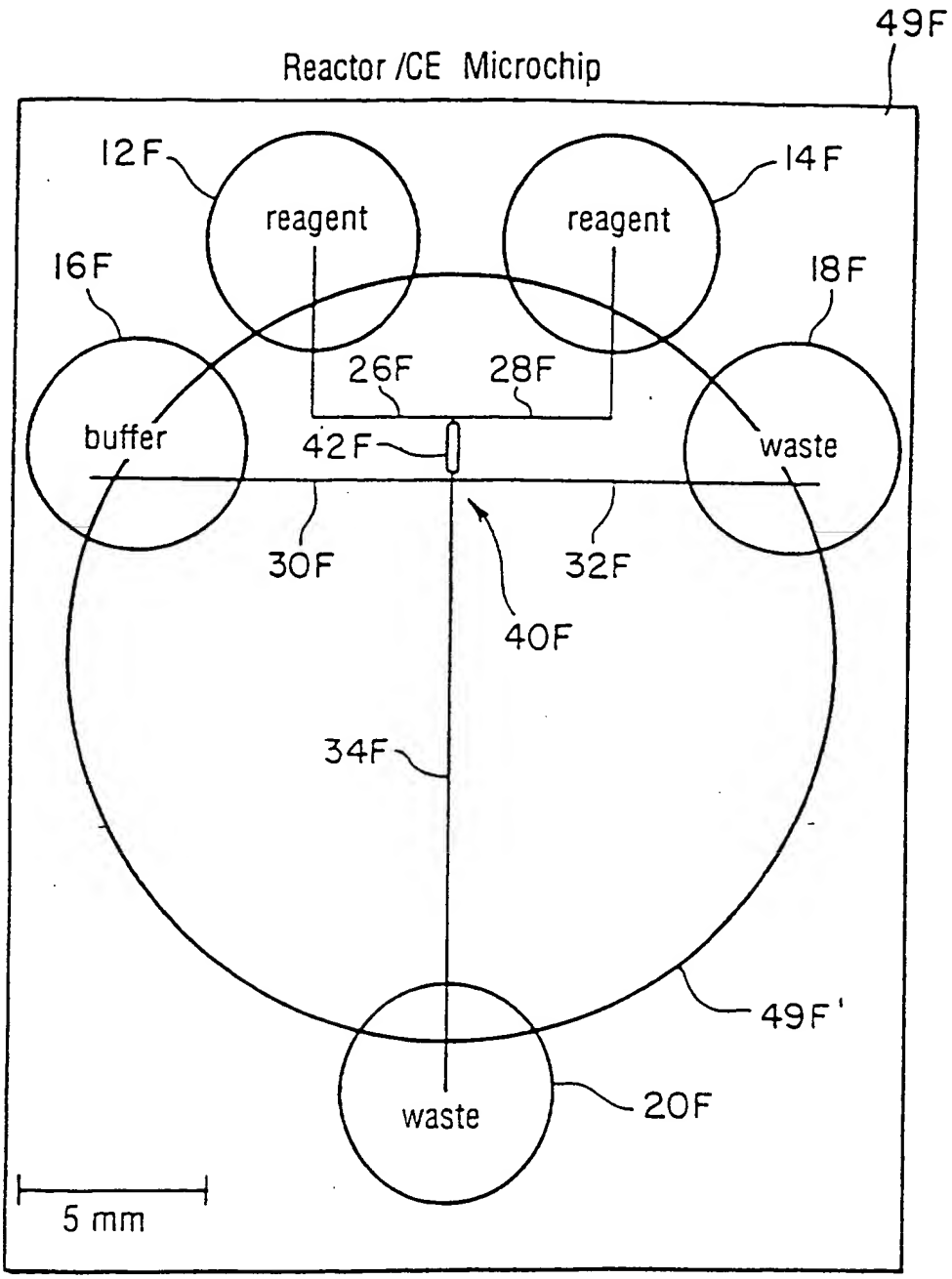

49F
Reactor /CE Microchip
12F
reagent
14F
reagent
16F
18F
26F
28F
buffer
42F
waste
30F
32F
40F
34F
49F'
waste
20F
5 mm

FIG. 28

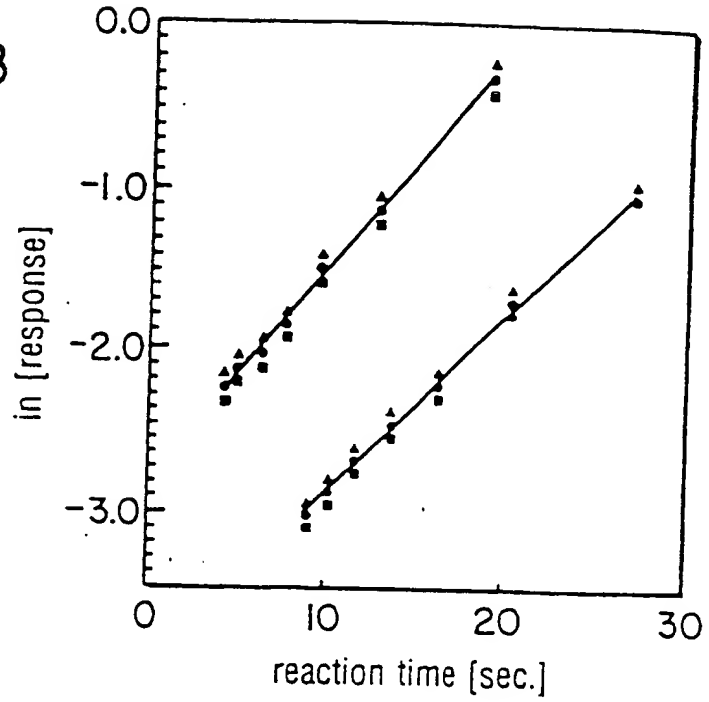

0.0
-1.0
-2.0
-3.0
ln [response]
0
10
20
30
reaction time [sec.]

FIG. 29

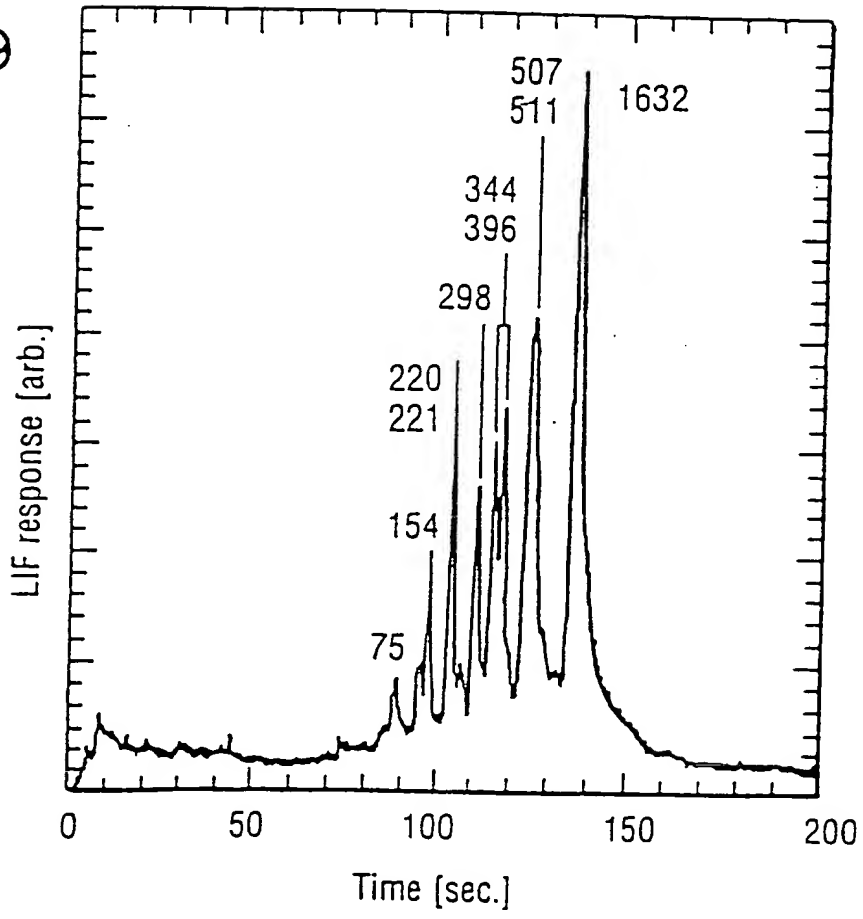

507
511
1632
344
396
298
220
221
154
75
LIF response [arb.]
0
50
100
150
200
Time [sec.]

FIG. 31(a)

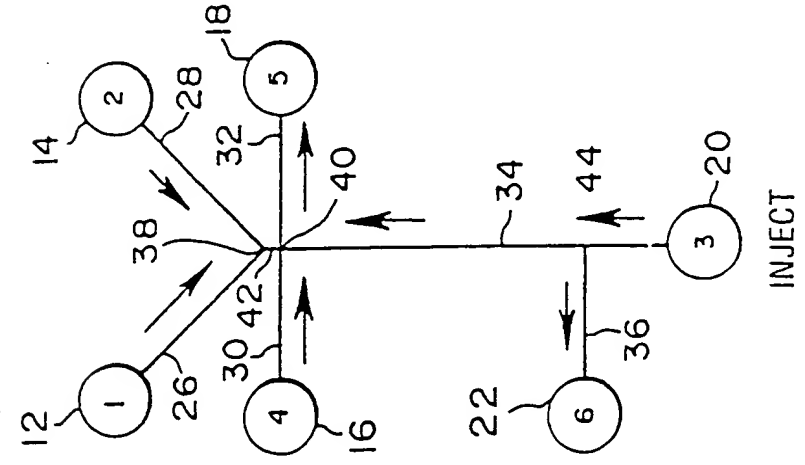


FIG. 31(b)

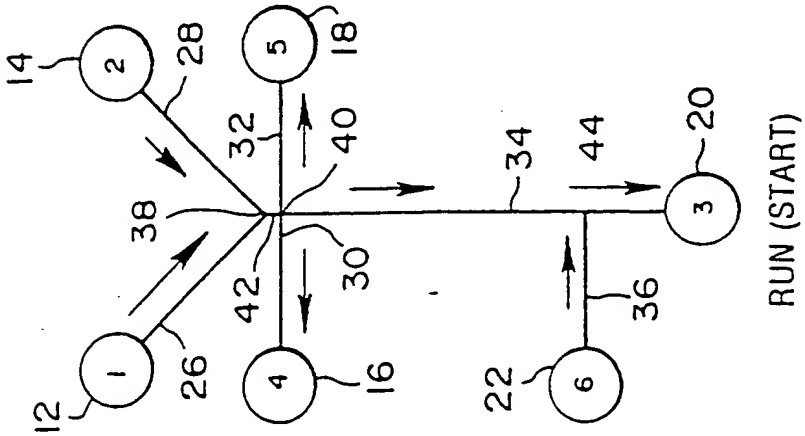
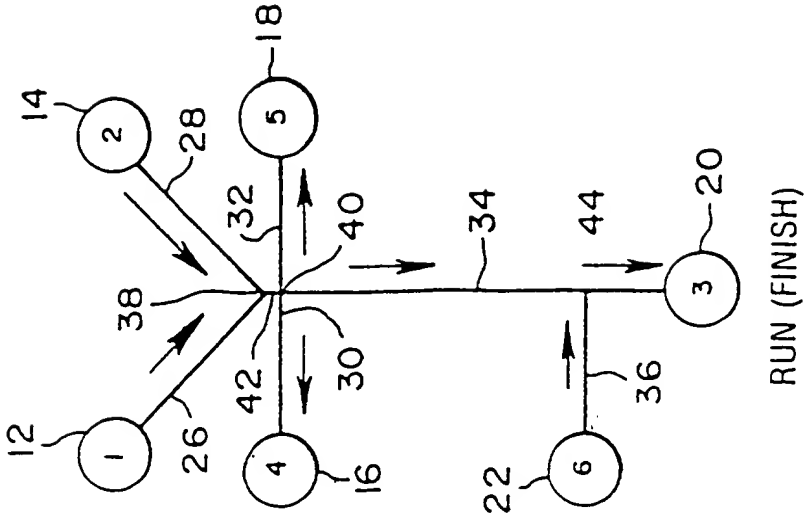


FIG. 31(c)



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/09492

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 27/00, 27/26

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/180; 422/99, 100, 101, 102, 103, 108, 110, 111; 204/299R, 180.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,908,112 (PACE) 13 March 1990, see entire document.	1-24
Y	US, A, 5,288,463 (CHEMELLI) 22 February 1994, see column 2, line 51 through column 5, line 20.	1-23
Y, P	US, A, 5,429,734 (GAJAR ET AL.) 04 July 1995, see entire document.	1-25
A	US, A, 5,229,297 (SCHNIPELSKY ET AL.) 20 July 1993, see entire document.	1-23

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 OCTOBER 1995	24 NOV 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ALEXANDER MARKOFF Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*